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ANIMAL SPERM PRESERVATION METHOD
[SPOSOB KONSERVIROVANIYA SPERMY ZHIVOTNYKH]

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(54) Animal sperm preservation method /1*

The invention pertains to the field of artificial insemination of agricultural animals, to methods of sperm preservation by the freezing method in particular.

A method of preserving the sperm of animals in a small storage cases, which includes dilution of the sperm to half the final stage of thinning with a protective medium, which contains 3% glycerin, cooling of the diluted semen to +4°C in 45-60 minutes, secondary dilution of the semen in a protective medium, which contains 11% glycerin, packaging in straw, equilibration and, finally, its freezing with subsequent thawing [1].

A shortcoming of this method is the rapid cooling of the semen to +4°C, the negative influence of the high concentration of glycerin and long contact time of the spermatozoa with it, which is manifested in damage to the apical edge of the acrosome, disturbance to stability of the lipid-protein complexes, and after freezing and thawing of the semen of the producer bulls mobility of the spermatozoa declines along with the absolute index of their survivability.

Moreover, during prolonged contact of the spermatozoa with the glycerin the leakage of a number of enzymes, amino acids, fatty acids and functional groups of cells of the spermatozoids into the plasma

*Number in the margin indicates pagination in the foreign text.

occurs, which leads to a reduction of the biological vitality of the sperm.

A method of preserving animal sperm is also known that includes its dilution in the first stage of a non-glycerin protective medium, and in the second stage of a protective medium with glycerin. In this case the cooling is carried out for two hours from 30 to 40°C, and the freezing is conducted at -80°C [2].

However, the prolonged contact (more than 30 minutes) of the spermatozoa with the glycerin lads to disturbance of the protein-lipid complex stability, morphological change of the acrosome, escape of enzymes into the plasma from the spermatozoa.

As a result of this action the activity of the spermatozoa after thawing is considerably lowered and reaches a level of approximately 2-2.5.

The aim of the invention is to increase the survival rate and biological vitality of the spermatozoids after freezing and thawing. /2

The goal is achieved due to the fact that the cooling of the sperm is carried out from +30 to +15°C at a rate of 0.4-0.5°C/minute, from +15 to +10°C at a rate of 0.3-0.4°C/minute and from +10° to +4°C at a rate of 0.2-0.3°C/minute, equilibration with glycerin is carried out at +4-+5°C for 25-30 minutes, and the freezing of the sperm is carried out in vapors of liquid nitrogen at temperature of -120—140°C for 8-8.5 minutes.

Example. The technology of the preparation and preservation of the semen of agricultural animals consists of the following. One first prepares a protective medium for diluting the semen. For the preparation one uses lactose, fructose, raffinose, magnesium sulfate, chicken egg yolk and Spermosan-3.

The dilution of semen with the aforementioned medium is conducted to half the final degree of dilution: the medium temperature at the time of dilution is +30°C.

Beakers with the diluted sperm are placed in a bath with water, whose temperature is +30°C, and they are transferred to a refrigerated case with constant temperature of +4°C.

Cooling of the semen is conducted in three stages: from +30 to +15°C, from +15 to +10°C and from +10 to +4°C. The cooling is carried out by adding pieces of melting ice or water at room temperature.

While conducting the experiment we employed several cooling modes and studied their effect on the biochemical and physiological indicators of the spermatozoa after freezing and thawing of the semen (see Table 1).

It was found that the optimal cooling mode is mode 3.

After cooling and holding the semen at +4°C for 3.0-3.5 hours the sperm was diluted a second time with the same protective medium, but one that contains additionally 10 ml of glycerin per 100 ml of distilled water.

The medium temperature at the time of the additional dilution was +4°C.

After the second dilution of the semen with the lactose-fructose-magnesium-egg yolk medium, which contains in addition glycerin, we carried out packaging of the semen manually or automatically in polymer straws with volume of 0.5 ml, held them in the glass case of the refrigerator for 30 minutes, after which the semen was immediately frozen.

The freezing is carried out in a wide-mouth Dewar flask, for which the straw is laid out horizontally on a special ramp and it is placed over vapors of liquid nitrogen at a level of 3-4 cm from the nitrogen level at the following temperatures (see Table 2).

The freezing time is eight minutes.

The semen freezing temperature is monitored with a thermocouple and regulated by the distance from the level of the liquid nitrogen to the ramp, where straw with the semen is placed horizontally.

The semen holding time in the liquid nitrogen vapors is eight minutes, after which the pieces straw are lowered into the liquid nitrogen, where they are kept for 30 days, and then they are transferred to a sperm bank for subsequent shipment to farms.

As a result of the semen contact with the glycerin for 30 minutes the biochemical and physiological indicators of the spermatozoa are improved, in particular the mobility of the spermatozoa and their life duration after thawing (see Table 3).

With the proposed cooling conditions one can achieve stabilization of the morphological, biochemical and physiological indicators of the semen (see Table 4).

The given indicators characterize the semen before its freezing.

Table 5 presents the quality indicators of the semen of the producer bulls after thawing.

The proposed method allows one to improve the quality of the thawed sperm, to increase the fertility of the animals with a reduction of the amount of sperm doses.

Table 1 Semen cooling conditions

Temperature and time Conditions	Semen cooling conditions, °C/min			
	1	2	3	4
From +30 to +15°C	0.2	0.3	0.4	0.5
From +15 to +10°C	0.2	0.2	0.3	0.4
From +10 to +4°C	0.1	0.1	0.2	0.3
The cooling time of semen To +4°C, minutes	160	135	85	63

Table 2
Influence of different conditions of producer bull semen freezing on mobility and vital duration of spermatozoa after thawing

/3

Experiment No.	Freezing temperature, °C	Indicator	Indicator	Indicator
		Mobility of spermatozoa after thawing, grade	Life duration of spermatozoa after thawing at 37°C, days	Absolute index of spermatozoa survival rate at +2-+4°C (s_a)
1	-120	4.6±0.21	8.8+0.2*	592.8±25.9*
2	-130	4.6±0.18	8.8+0.2*	593±24.5*
3	-140	4.6±0.21	9.0+0.3*	590±26.9
4	-160	4.2±0.35	7.6+0.5	446±17.1

- * - Differences are statistically reliable with respect to the prototype.

Table 3

Время выдержки разбавленного семени средой, содержащей глицерин, мин (a)	Подвижность живчиков после оттаивания в зависимости от режимов охлаждения (b)				Продолжительность жизни живчиков после оттаивания в зависимости от режимов охлаждения (c)				Устойчивость белково-холестериновых комплексов в зависимости от времени выдержки, мкг холестерина на 10^9 живчиков (d)	
	2	3	4	контроль (e)	2	3	4	контроль (e)	3	контроль (e)
1^I	4,3±0,3	4,3+0,1	4,2±0,09	4,25±0,1	7,4±0,2	7,7±0,2	7,0±0,3	7,0±0,2	166±6,8	146+7,2
5^I	4,2±0,1	4,25±0,07	4,0±0,08	-	7,0±0,3	7,3±0,3	7,0±0,25	-	148±7,8	-
10^I	4,25±0,12	4,25+0,1	4,15±0,2	-	7,0±0,2	7,3+0,3	6,9+0,25	-	150±8,1	-
15^I	4,2±0,15	4,25±0,2	4,2±0,1	-	7,2±0,2	7,3±0,2	7,2±0,18	-	155±7,7	-
20^I	4,25±0,12	4,35±0,1	4,3±0,08	-	7,5±0,25	7,5±0,2	7,3±0,3	-	167±8,0	-
25^I	4,3±0,15	4,5±0,12	4,35±0,14	-	7,4±0,3	7,8±0,2*	7,4±0,2	-	174±3,4*	-
30^I	4,4±0,12	4,6±0,1*	4,35±0,12	-	7,6±0,2	7,8±0,2*	7,4±0,3	-	178±6,2*	-
35^I	4,2±0,1	4,3±0,14	4,25±0,12		7,3±0,3	7,5±0,3	7,4±0,3	-	171±7,4	-
40^I	4,1±0,15	4,25±0,1	4,15±0,08	-	7,1±0,2	7,4±0,3	7,2±0,2	-	162±6,5	-

*Differences are statistically reliable with respect to the prototype.

Table 3
Influence of semen holding time after additional dilution of semen in a synthetic medium, which contains glycerin, on the biochemical and physical indicators of the spermatozoa.

Key:
(a) Holding time Of diluted semen In a medium that Contains glycerin, Minutes;
(b) Mobility of spermatozoa after thawing depending on cooling conditions;
(c) Life duration of spermatozoa after thawing depending on cooling conditions;
(d) Stability of protein- cholesterol complexes depending on Holding time, Mcg of Cholesterol Per 10^9 Spermatozoa;
(e) Control

Table 4
Morphological, biochemical and physiological indices of semen prior to thawing. /5

Indicator	Proposed method of semen cooling	British patent number 1,479,648
Resistance of protein-cholesterol complexes, mcg of cholesterol per 10^9 spermatozoa	221±6.7*	203±5.8
Resistance of protein-phospholipid complexes, mcg % phospholipids	2195±17.2*	2022±15.2
% spermatozoa with normal acrosome	75±1.5*	9.0±1.3
Activity of the glutamine-asparagin transaminase in the semen plasma	161±2.5	184±2.8**
Mobility of spermatozoa	8.0±0.08*	7.5±0.06

XX - $P<0.01$;
X - $P<0.05$.

Table 5
Morphological, biochemical and physiological indices of semen prior to thawing.

Experiment, Number	Indicator	Proposed method
1	Mobility of spermatozoa after thawing	4.75±0.09
2	Life duration of spermatozoa after thawing at 37°C, hours	8.8±0.34
3	Absolute index of spermatozoa survival rate after thawing at 37°C	30.2±1.2
4	Life duration of spermatozoa after thawing at +2-+4°C	212.0±7.3
5	Absolute index of spermatozoa survival rate after thawing at +2-+4°C	602.6±15.4
6	Condition of spermatozoa acrosome: Normal Destroyed	 61.5±1.2 38.5±1.2
7	Stability of the protein-cholesterol complexes, mcg of cholesterol for 10 spermatozoa	185
8	Fertility of cows from first insemination	67.5

CLAIMS

The method of animal sperm preservation, which includes its dilution with a non-glycerin protective medium, cooling, repeated dilution of the sperm by a protective medium, which contains glycerin, equilibration with glycerin and freezing, characterized by the fact that in order to improve the vital functioning and biological value of the spermatozoids after freezing and thawing, the cooling of the sperm is carried out from +30 to +15°C at a rate of /6
0.4-0.5°C/minute, from +15 to +10°C at a rate of 0.3-0.4°C/minute, and from +10 to +4°C at a rate of 0.2-0.3°C/minute, equilibration

with glycerin is carried out at +4-+5°C for 25-30 minutes, and the sperm freezing is carried out in vapors of liquid nitrogen at temperature of -120 - 140°C for 8-8.5 minutes.

Information sources considered by the examining board:

1. The French Straw technique, 1976.
2. British patent number 1,479,648, Intl. Class A 01 N 1/202, 1977 (prototype).